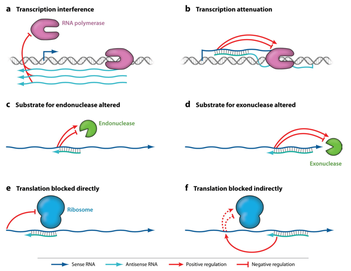
Putative regulatory role of *GlyS* antisense RNA in an obligate insect symbiont *Buchnera aphidicola*

Siyuan (Lily) Cheng and Allison K. Hansen
Department of Entomology, School of Integrative Biology, University of Illinois at Urbana-Champaign

Introduction

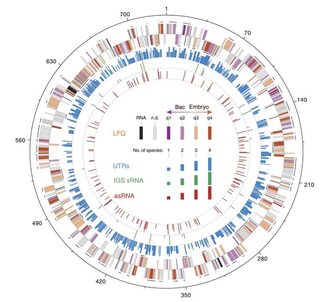
Small RNAs (sRNAs) are recognized for their importance in the regulation of gene expression within all domains of life. One type of small RNA, antisense RNAs (asRNA), are single-stranded RNAs that are expressed complementary to messenger RNAs (mRNA), and are known to regulate gene expression at either the transcriptional or translational levels in bacteria (Figure 1). Despite the prevalence of these non-coding RNAs, the putative regulatory roles of many identified sRNAs in bacteria are largely unexplored. Further research on sRNAs will lead to novel insights into the regulation of genes in bacteria.



Thomason MK, Storz G. 2010.
Annu. Rev. Genet. 44:167–88

Figure 1. Mechanisms by which sRNAs act

A fascinating system for uncovering the regulatory roles of asRNAs in is bacterial genomes that have been greatly reduced in genome size compared to their free living relatives. Reduced bacterial genomes are generally observed in intracellular symbionts, pathogens, and organelles and result in the loss of most canonical regulatory genes, because of genetic drift. For example, an uncultivable symbiont with a small genome (*Buchnera aphidicola*) that is harbored in the pea aphid (*Acyrthosiphon pisum*) has lost nearly all transcription factors, and its operons have been fragmented. Nevertheless, a recent study uncovered widespread expression of conserved antisense sRNAs in five reduced *Buchnera* genomes that diverged an estimated 65 million years ago (Figure 2). It is unknown whether or not these sRNAs play regulatory roles in *Buchnera*, especially since *Buchnera* exhibits differential protein expression at different *Buchnera* life stages at the protein level.

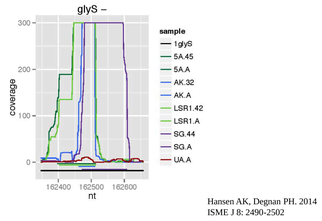


Hansen AK, Degnan PH. 2014
ISME J 8: 2490-2502

Figure 2. Genome-wide map of protein and sRNA expression in four *Buchnera* taxa.

Objective

The broad objective of this research project is to determine the regulatory role of the conserved sRNAs in gene expression of *Buchnera*. The antisense RNA of *GlyS* is the first candidate for investigation because it is highly conserved and expressed and its structure is thermodynamically stable. *GlyS* is the gene that encodes glycyl-tRNA synthetase subunit beta.



Hansen AK, Degnan PH. 2014
ISME J 8: 2490-2502

Figure 3. Expression of the antisense RNA (as*GlyS*) in different lineages of *Buchnera* (5A, AK, LSR1, SG, and UA)

Method

As *Buchnera* is an uncultivable bacterium, we are investigating the regulation of the *GlyS* gene by the asRNA with a dual plasmid vector system in *Escherichia coli (E. coli)* with one plasmid encoding the asRNA and the other the coding sequence (CDS) fused to a green fluorescent protein (GFP) gene. The asRNA is expected to either inhibit or activate the translation of the CDS. The effect will be reported by the intensity of fluorescence measured (Figure 4).

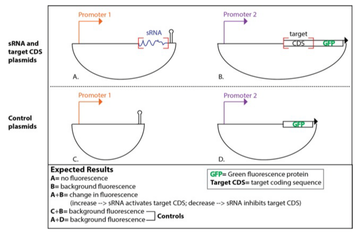


Figure 4. Dual plasmid vector system used to investigate the regulatory role of the *GlyS* asRNA

To construct the dual plasmid vector system, the asRNA and the CDS is ligated into restriction-digested vector. Both plasmids are transformed into *E. coli* cells respectively. Plasmids are then extracted and screened for the insert with PCR and small-scale digestion. Plasmids are sequenced to confirm the integration of target sequence. Then the two plasmids are double-transformed into the same *E. coli* cells.

Progress

Construction of the asRNA plasmid: The asRNA adapter molecule (~ 100 bp) was ligated into a restriction-digested vector. The plasmid was transformed into *E. coli* cells. Cells were grown and plasmids extracted, which were then used for small-scale test digestion screening for the insert. The asRNA insert was confirmed by plasmid Sanger Sequencing (Figure 5).

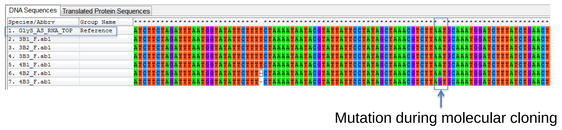


Figure 5. Plasmid sequencing result aligned with *GlyS* asRNA reference sequence

Construction of the CDS plasmid: The CDS (~ 2 kb) was PCR amplified after the annealing temperature was optimization by gradient PCR. The PCR product was restriction-digested and ligated into a restriction-digested and dephospohorylated vector with GFP. The plasmid was transformed into *E. coli* cells. The cells were screened by PCR with primers designed to amplify the CDS. Plasmids were extracted for small-scale test digestion. The CDS insert was confirmed with plasmid Sanger Sequencing with a plasmid primer and CDS forward and reverse primers (Figure 6).

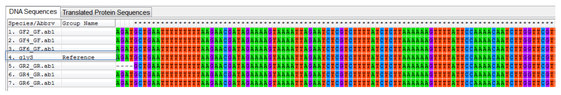


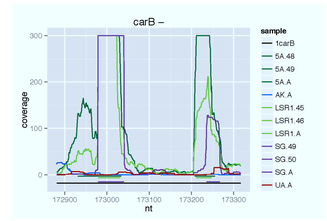
Figure 6. Plasmid sequencing results assembled and aligned with the *GlyS* CDS reference sequence

Double transformation: in progress. The selectable marker for the asRNA plasmid is chloramphenicol antibiotic resistance; the selectable marker for CDS plasmid is the ampicillin antibiotic resistance. Colonies are being currently screened with primers specific for each plasmid.

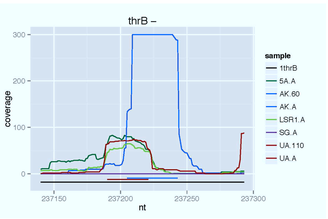
Next Steps

- ✓ Complete double transformation
- ✓ Establish negative control
- ✓ Make fluorescence measurement
- ✓ Obtain results for the first candidate sRNA

Investigate the regulatory role of more antisense sRNAs (Figure 7).



carB: carbamoyl phosphate synthase large subunit



thrB: homoserine kinase

Figure 7. Some asRNA candidates for future investigation

Acknowledgments

We thank members of the Hansen lab and the Degnan lab for technical assistance and helpful suggestions. The vectors were prepared by the Vanderpool lab. This project is supported by the Fall 2015 and Spring 2016 LAS James Scholar Preble Research Award to SC and UIUC start-up funding and AFRI USDA funding to AKH.